Training Program

Interpretation of Results

Your lab has measured two different biomarkers associated with the progression to preeclampsia with severe features. sFlt-1 (soluble fms-like tyrosine kinase 1) and PlGF (placental growth factor) in serum or plasma (K2 EDTA) are both associated with placental dysfunction during pregnancy. These are each measured then a ratio of the two biomarkers is calculated. The ratio is the sFlt-1 concentration divided by the PlGF concentration (both expressed in the same units, pg/mL). The ratio is a unitless number, reported with 4 significant digits when below 1000 and as a whole number above 1000.

That ratio (B·R·A·H·M·S™ sFlt-1 KRYPTOR™ to B·R·A·H·M·S PlGF plus KRYPTOR) is to be used along with other laboratory tests and clinical assessments to aid in the risk assessment of pregnant women (singleton pregnancies between gestational age 23+0 to 34+6/7weeks) hospitalized for hypertensive disorders of pregnancy (preeclampsia, chronic hypertension with or without superimposed preeclampsia, or gestational hypertension) for progression to preeclampsia with severe features (as defined by American College of Obstetricians and Gynecologists (ACOG) guidelines[1]) within 2 weeks of presentation.

For the sFlt-1/PlGF ratio, the clinical cut-off was established in the PRAECIS clinical study (NCT03815110). The cut-off of 40 was derived in a derivation cohort of 220 patients. This cut-off was then validated in a validation cohort of 556 patients[2].

sFlt-1/PlGF ≥ 40 (high risk):

If the result of the ratio is higher or equal to 40, the pregnant woman is at high risk for progression to preeclampsia with severe features within 2 weeks.

sFlt-1/PlGF < 40 (low risk):

If the result of the ratio is lower than 40, the pregnant woman is at low risk for progression to preeclampsia with severe features within 2 weeks.

Low risk for progression to preeclampsia with severe features within 2 weeks:

The woman at low risk for progression to preeclampsia with severe features within 2 weeks would receive standard of care including expectant management according to the ACOG guidelines. A woman with “false negative” result would continue receive existing standard of care including monitoring of future signs of preeclampsia with severe features.

High risk for progression to preeclampsia with severe features within 2 weeks:

The woman at high risk for progression to preeclampsia with severe features within 2 weeks would receive stepped up care according to the ACOG guidelines. The elevation in the sFlt-1/PlGF ratio antedates ACOG-defined criteria for delivery (e.g., LFT elevations, thrombocytopenia abnormal umbilical Doppler), and therefore is useful to step-up appropriate care and intensify surveillance before severe features develop. A woman with “false positive” result would receive stepped up care and would not be harmed by additional monitoring.

The sFlt-1/PlGF ratio has been validated specifically for predicting risk that preeclampsia will progress to severe features within 2 weeks of presentation. It has not been validated for any other purpose. The test is not intended to replace standard of care recommendations defined by the ACOG standard.

- **Do not use the sFlt-1/PlGF ratio test for making a diagnosis of preeclampsia or preeclampsia with severe features.**
- **Do not use the sFlt-1/PlGF ratio test as a stand-alone test for monitoring of hypertensive disorders of pregnancy.**
- **sFlt-1/PlGF ratio ≥ 40 should not be used to aid in the decisions of pregnancy delivery.**

- **sFlt-1/PlGF ratio < 40 still requires standard of care per ACOG guidelines.**
- **sFlt-1/PlGF ratio should not be used to aid in decision of hospital discharge.**
- **sFlt-1/PlGF ratio is not intended to inform the healthcare provider whether or not to change treatment, including medication or hospitalization.**

A link to the full instructions for use can be found at: ifu.brahms.de

Warnings and Precautions – Test Interpretation

The sFlt-1/PlGF ratio is indicated to be used as an aid in the management of the patient and is a prognostic assay intended to stratify hospitalized patients in two risk groups (low risk and high risk of progression to preeclampsia with severe features within two weeks from presentation). The assay results should only be used in conjunction with information available from clinical evaluations and other standard of care procedures. The test result is not to be used to replace clinical judgement.

B·R·A·H·M·S sFlt-1 KRYPTOR must be run in conjunction with B·R·A·H·M·S PlGF plus KRYPTOR and the same patient sample must be used to run both assays. B·R·A·H·M·S sFlt-1 KRYPTOR and B·R·A·H·M·S PlGF plus KRYPTOR are not intended to be used individually.

The clinical cutoff of the sFlt-1/PlGF ratio should not be used from other manufacturer's assays. Use of another manufacturer's clinical cutoff may result in erroneous results; for example false positive or false negative.

B·R·A·H·M·S sFlt-1 KRYPTOR and B·R·A·H·M·S PlGF plus KRYPTOR should not be used for a woman with a multiple pregnancy because the safety and effectiveness of the device has not been established in pregnant women with a multiple pregnancy (i.e. pregnancy with more than one fetus).

B·R·A·H·M·S sFlt-1 KRYPTOR and B·R·A·H·M·S PlGF plus KRYPTOR should not be used for a woman receiving intravenous heparin within 24 hours of testing because the safety and effectiveness of the device has not been established in pregnant women who received intravenous heparin within 24 hours of testing of the device.

B·R·A·H·M·S sFlt-1 KRYPTOR and B·R·A·H·M·S PlGF plus KRYPTOR should not be used for a woman receiving exogenous PlGF-2 or PlGF-3 for therapeutic use at concentration higher than 100 pg/mL because the safety and effectiveness of the device has not been established in pregnant women who received exogenous PlGF-2 or PlGF-3 for therapeutic use at concentration higher than 100 pg/mL. The B·R·A·H·M·S PlGF plus KRYPTOR assay was found in bench studies to be impacted by PlGF-2. With samples at equal levels of PlGF (i.e. PlGF-1) and PlGF-2, the reported concentration is within 1% compared to samples without PlGF-2. The normal endogenous level of PlGF-2 in samples collected from pregnant women has not been established.

The clinical management of each patient should be dependent on the patient's health care provider's recommendations as inferred from their clinical status. Therefore, the sFlt-1/PlGF ratio should not be used as a deciding factor to change management plans, especially discharge from hospital or delivery decisions.

Reference Range

In a population of 166 healthy pregnant women between week 23+0 up to week 34+6, sFlt-1 median was established at 1,299 pg/mL, the 2.5th percentile at 526 pg/mL and the 97.5% percentile at 5,140 pg/mL and the PlGF median at 450 pg/mL, the 2.5th percentile at 87 pg/mL and the 97.5% percentile at 1,202 pg/mL. The sFlt-1/PlGF ratio median was established at 3.16, the 2.5th percentile at 0.84 and the 97.5% percentile at 31.1.

Clinical Study Data

Based on the data collected during the PRAECIS clinical study (NCT03815110)[2] and subsequent analysis the following conclusions were derived:

The prognostic performance of the sFlt-1/PlGF ratio with a cut-off of 40 established during Part 1 of the study was successfully validated to predict preeclampsia with severe features within two weeks of hospitalization during Part 2 of the Clinical Trial. The specific data for prognostic performance estimates and 95% CI are summarized in Table 1.

Table 1: Prognostic performance estimates (sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV)) and 95% confidence intervals (CI) of the sFlt-1/PlGF ratio with a cut off at 40 for the development of severe features of PE within 2 weeks (primary endpoint).

	N	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
PE with severe features within 2 weeks of hospitalization*	556	93.5% (89.1- 96.3)	74.9% (70.2 - 79.0)	65.2% (59.3- 70.6)	95.8% (92.9 - 97.6)

* *Severe features of PE: Systolic blood pressure of 160 mm Hg or higher, or diastolic blood pressure of 110 mm Hg or higher on two occasions at least 4 hours apart while the patient is on bed rest (unless antihypertensive therapy is initiated before this time, Thrombocytopenia (platelet count less than 100,000/microliter), impaired liver function as indicated by abnormally elevated blood concentrations of liver enzymes (to twice normal concentration), severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for my alternative diagnoses, or both, progressive renal insufficiency (serum creatinine greater than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease), pulmonary edema, new-onset cerebral or visual disturbances, headache unresponsive to medication and not accounted for by alternative diagnoses. Women with gestational hypertension or chronic hypertension who developed new evidence of thrombocytopenia, impaired liver function, renal insufficiency, pulmonary edema, visual loss or cerebral disturbances as described above were considered to have preeclampsia with severe features*[2].

False positives
Based on the clinical validation study, the positive predictive value of the test is 65%. This may be understood that with a high risk result, 35% of patients with a positive (i.e. high risk) result did not develop preeclampsia with severe features within two weeks from testing.

False negatives
Based on the clinical validation study, the negative predictive value of the test is 96%. This may be understood that with a low risk result, 4% of the patients with a negative (i.e. low risk) result did develop preeclampsia with severe features within two weeks from testing.

The prognostic performance of the numeric sFlt-1/PlGF ratio to predict development of PE with severe features within two weeks of hospitalization was statistically significantly and substantially higher than the prognostic performance of other commonly used clinical (highest SBP, highest DBP) and laboratory markers (AST, ALT, Creatinine, platelets) considered individually.

Impact of hypertensive disorder at presentation on the prognostic performance of the device:

Hypertensive disorders	n	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Preeclampsia	142	96.3% (89.7 - 98.7)	63.9 (51.4 - 74.8)	78.0% (68.9 – 85.0)	92.9% (81.0 - 97.5)
Superimposed Preeclampsia	68	93.8% (79.9 - 98.3)	55.6% (39.6- 70.5)	65.2% (50.8 - 77.3)	90.9% (72.2 - 97.5)
Chronic Hypertension	232	91.8% (80.8 - 96.8)	80.3% (74.0 - 85.4)	55.6% (44.7 - 65.9)	97.4% (93.4 – 99.0)
Gestational Hypertension	114	87.5% (69.0 - 95.7)	78.9% (69.4- 86.0)	52.5% (37.5 - 67.1)	95.9% (88.7 - 98.6)

Impact of maternal age at presentation on the prognostic performance of the device:

Maternal age	n	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
<35 years	370	93.2% (87.5 - 96.4)	79.0% (73.4 - 83.7)	71.1% (63.9 - 77.3)	95.4% (91.5 - 97.6)
≥35 years	186	94.4% (84.9 - 98.1)	67.4% (59.0 - 74.8)	54.3% (44.2 – 64.0)	96.7% (90.8 - 98.9)

Impact of gestational age at presentation on the prognostic performance of the device:

Gestational age	n	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
<30 weeks	188	98.6% (92.7 - 99.9)	80.7% (72.5 - 86.9)	76.8% (67.4 - 84.2)	98.9% (94.2 - 99.9)
≥30 weeks	368	90.2% (83.3 - 94.4)	72.3% (66.5 - 77.4)	58.7% (51.3 - 65.8)	94.4% (90.2 - 96.8)

From the data collected during the PRAECIS clinical study (NCT03815110), the sFlt-1/PlGF ratio were found to be between 0.38 and 7,367.

Interfering Substances

Interference/cross-reactivity was determined following CLSI Guideline EP7-A3 and EP37. The substances evaluated with the B·R·A·H·M·S sFlt-1 KRYPTOR and the B·R·A·H·M·S PlGF plus KRYPTOR were found not to affect the test performance and the sFlt-1/PlGF ratio at the concentrations shown below:

Interfering Substance	**Interferent Concentration**	**Interference**
Albumin	60 g/L	No interference up to 60 g/L
Bilirubin	25 mg/dL	No interference up to 250 mg/L
Hemoglobin	5 g/L	No interference up to 5 g/L
Triglycerides	30 g/L	No interference up to 30 g/L
Acetaminophen	200 mg/L	No interference up to 200 mg/L
Acetylsalicylic Acid	652 mg/L	No interference up to 652 mg/L
L-Ascorbic acid	30 mg/L	No interference up to 30 mg/L
Caffeine	59 mg/L	No interference up to 59 mg/L
Calcium	200 mg/L	No interference up to 200 mg/L
Dihydralazin	0.2 g/L	No interference up to 0.2 g/L
Ethanol	5% (v/v)	No interference up to 5% (v/v)
Folic Acid	2.4 mg/L	No interference up to 2.4 mg/L
Gentamicin	10 mg/L	No interference up to 10 mg/L
Heparin	3,000 U/L	No interference up to 3000 U/L *
Ibuprofen	500 mg/L	No interference up to 500 mg/L
Iron	72 mg/L	No interference up to 72 mg/L
α-Methyldopa	15 mg/L	No interference up to 15 mg/L
Metoprolol	5 mg/L	No interference up to 5 mg/L

* see warning above for women receiving intravenous heparin

Cross-Reactant	**Cross-Reactant Concentration**	**Cross Reactivity**
Recombinant Human PlGF-1*	10,000 pg/mL	No cross reactivity
Recombinant Human PlGF-2	100 pg/mL	No cross reactivity
Recombinant Human PlGF-3	100 pg/mL	No cross reactivity
Recombinant Human VEGF A	5,000 pg/mL	No cross reactivity

Recombinant Human VEGF B	5,000 pg/mL	No cross reactivity
Recombinant Human VEGF/PlGF Heterodimer	10,000 pg/mL	No cross reactivity

* only applicable to B·R·A·H·M·S sFlt-1 KRYPTOR

Bibliography

1. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222.Obstet Gynecol, 2020; 135(6): p. e237-e260
2. Thadhani et al., Circulating Angiogenic Factor Levels In Hypertensive Disorders of Pregnancy. NEJM, 2022;1 (12), EVIDoa2200161

Revision History

Date: [2023-08]
(This version supersedes all earlier instruction manuals.)

Date of Revision	Version	Description of Changes
[2023-05]	Version1	Initial release
[2023-08]	Version2	Add revision history, footer, remove patent link, update sentence on the ratio unit number

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